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Nanosizing — Oral formulation development and biopharmaceutical evaluation ☆

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Abstract

Poor aqueous solubility represents a major hurdle in achieving adequate oral bioavailability for a large percentage of drug compounds in drug development nowadays. Nanosizing refers to the reduction of the active pharmaceutical ingredient (API) particle size down to the sub-micron range, with the final particle size typically being 100–200 nm. The reduction of particle size leads to a significant increase in the dissolution rate of the API, which in turn can lead to substantial increases in bioavailability. This review describes the principles behind nanosizing, the production and characterization of nanoformulations as well as the current experience with utilization of such formulations *in vivo*.

Keywords: Nanosizing; Nanoparticle; Nanosuspension; Bioavailability enhancement; Dissolution; Formulation

Contents

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1. Introduction

Advances in combinatorial chemistry, biology and genetics in the recent years have led to a steady increase in the number of drug candidates under development. Due to the phospholipidic nature of cell membranes, a certain degree of lipophilicity is oftentimes a requirement for the drug compound, not only to be absorbed through the intestinal wall following oral administration but possibly also to exert its pharmacological action in the target tissue. While high lipophilicity is advantageous in terms of compound permeability, it intrinsically translates into poor aqueous solubility. Since the first step in the oral absorption process is dissolution of the drug compound in the gastrointestinal lumen contents, poor aqueous solubility is rapidly becoming the leading hurdle for formulation scientists working on oral delivery of drug compounds [1].

Nanosizing refers to the reduction of the active pharmaceutical ingredient (API) particle size down to the sub-micron range. While reduction of particle size has been employed in pharmaceutical industry for several decades, recent advances in milling technology and our understanding of such colloidal systems have enabled the production of API particles of 100–200 nm size in a reproducible manner. The sub-micron particles are stabilized with surfactants or polymers in nanosuspensions which can be further processed into standard dosage forms, such as capsules or tablets, suitable for oral administration. These nanoformulations offer increased dissolution rates for drug compounds and complement other technologies used to enhance bioavailability of insoluble compounds (BCS Class II and IV) such as solubility enhancers (*i.e.* surfactants), liquid-filled capsules or solid dispersions of drugs in their amorphous state.

The advantages of nanoformulations in oral drug delivery have been demonstrated *in vitro* in dissolution testing and *in vivo* in both preclinical studies as well as clinical trials. Nanocrystalline API has been shown to dramatically increase the rate of dissolution *in vitro*, improve bioavailability, reduce variability and alleviate positive food effects for orally administered drug molecules. As seen in Table 1, there are currently five pharmaceutical products that utilize nanocrystalline API to achieve their drug delivery goals. The goal of this review is to cover the theoretical background and practical aspects behind utilization of nanosizing as a means to improve oral bioavailability of drug compounds. We discuss development of nanosized formulations through the different stages of drug development covering both formulation aspects such as excipient selection and assessment of bioperformance. Examples of utilization of nanosizing to increase oral absorption are provided and, when applicable, compared to other novel oral dosage forms. Finally, we discuss the advantages and limitations of nanosizing in terms of its applicability in the drug development process.

2. Increasing dissolution rate through nanosization — theoretical aspects

The solid API dissolution rate is proportional to the surface area available for dissolution as described by the Nernst–Brunner/Noyes–Whitney equation [2–4]:

$$\frac{dX}{dt} = \frac{A \cdot D}{h}\left(C_s - \frac{Xd}{V}\right) \quad (1)$$

where dX/dt=dissolution rate, Xd=amount dissolved, A=particle surface area, D=diffusion coefficient, V=volume of fluid available for dissolution, C_s=saturation solubility, h=effective boundary layer thickness.

Based on this principle, API micronization has been extensively used in the pharmaceutical industry to improve oral bioavailability of drug compounds. It is evident that a further decrease of the particle size down to the sub-micron range will further increase dissolution rate due to the increase of the effective particle surface area [5]. For example in the case of aprepitant, the nanocrystal dispersion of 120-nm particle size exhibits a 41.5-fold increase in surface area over the standard 5 μm suspension [6]. Furthermore, as described by the Prandtl equation, the diffusion layer thickness (h) will also be decreased thus resulting in an even faster dissolution rate [7].

In addition to the dissolution rate enhancement described above, an increase in the saturation solubility of the nanosized API is also expected [8], as described by the Freundlich–Ostwald equation:

$$S = S_\infty \exp\left(\frac{2\gamma M}{r\rho RT}\right) \quad (2)$$

where S=saturation solubility of the nanosized API, S_∞=saturation solubility of an infinitely large API crystal, γ is the crystal-

Table 1
Current marketed pharmaceutical products utilizing nanocrystalline API

Product	Drug compound	Indication	Company	Nanoparticle technology
RAPAMUNE®	Sirolimus	Immunosuppressant	Wyeth	Elan Drug Delivery Nanocrystals®
EMEND®	Aprepitant	Antiemetic	Merck	Elan Drug Delivery Nanocrystals®
TriCor®	Fenofibrate	Treatment of hypercholesterolemia	Abbott	Elan Drug Delivery Nanocrystals®
MEGACE® ES	Megestrol acetate	Appetite stimulant	PAR Pharmaceutical	Elan Drug Delivery Nanocrystals®

medium interfacial tension, M is the compound molecular weight, r is the particle radius, ρ is the density, R is a gas constant and T is the temperature.

Assuming a molecular weight of 500, $\rho = 1$ g/mL and a γ value of 15–20 mN m^{-1} for the crystal-intestinal fluid interfacial tension, the above equation would predict an approximately 10–15% increase in solubility at a particle size of 100 nm. However a more significant increase in solubility appears to occur in reality *e.g.* Muller and Peters reported an increase of 50% in the solubility of an insoluble antimicrobial compound when the particle size was reduced from 2.4 μm to 800 or 300 nm [8]. This increase in solubility leads to a further increase in dissolution rate and, as a result, nanosuspensions often achieve significantly higher exposure levels compared to suspensions of micronized API, even when the same surfactants are used. Finally, the increase in surface wetting by the surfactants in the nanosuspension formulations most likely results in a further enhancement of the dissolution rates compared to micronized suspensions.

3. Formulation development of nanoformulations

Compared with formulation efforts using traditional processes such as wet-granulation (WG), roller-compaction (RC), or direct compression (DC), development of nanoformulations is one of the more complex formulation works. Not only must the drug particles be rendered into nanosized domains *via* technically demanding processes, but they must also be stabilized and formulated rigorously to retain the nature and properties of the nanoparticles. This review will focus on Elan's nanomilling technology for oral formulation applications. Before delving in, a snapshot of other nanoparticle technologies is provided.

For the purposes of this discussion, the definition of "nanoparticles" will be confined to crystalline particles with a monolithic core. There are two main approaches to making nanoparticles: 'top down' and 'bottom up' technologies [9,10]. The 'top down' approach is by far the more popular; it will be referred to as 'nanosizing'. The approach basically relies on mechanical attrition to render large crystalline particles into nanoparticles. Examples of the 'top down' approach include Elan's NanoCrystal® wet-milling technology [11] and SkyePharma's Dissocubes® high-pressure homogenization technology [9,12]. The 'bottom up' approach relies on controlled precipitation/crystallization [10]. The process involves dissolving the drug in a solvent and precipitating it in a controlled manner to nanoparticles through addition of an anti-solvent (usually, water). This technology is available from DowPharma (Midland, MI, USA) and BASF Pharma Solutions (Florham Park, NJ, USA). A hybrid approach is also feasible. Baxter's NANOEDGE® technology employs both 'bottom up' and 'top down' approaches through microprecipitation and homogenization [9].

The focus and examples of this review will be based on the application of the NanoCrystal® technology to the development of nanoformulations. However, most of the discussion on properties and characterization of nanoparticles, selection of stabilizers, and considerations in nanoformulation development

3.1. Selection of excipients

Formulation of nanosuspension requires a careful selection of stabilizers. Stabilizers are needed to stabilize the nanoparticles against inter-particle forces and prevent them from aggregating. At the nanometer domain, attractive forces between particles, due to dispersion or van der Waals forces, come into play [13]. This attractive force increases dramatically as the particles approach each other, ultimately resulting in an irreversible aggregation. To overcome the attractive interaction, repulsive forces are needed. There are two modes of imparting repulsive forces or energetic barriers to a colloidal system — steric stabilization and electrostatic stabilization. Steric stabilization is achieved by adsorbing polymers onto the particle surface. As the particles approach each other, the osmotic stress created by the encroaching steric layers acts to keep the particles separate. Electrostatic stabilization is obtained by adsorbing charged molecules, which can be ionic surfactants or charged polymers, onto the particle surface. Charge repulsion provides an electrostatic potential barrier to particle aggregation. Typically, the use of steric stabilization alone is sufficient to stabilize the nanoparticles and prevent irreversible aggregation. However, enough attractive force between particles may still remain to cause a loose and reversible flocculation. To circumvent flocculation, steric stabilization is often combined with electrostatic stabilization for additional repulsive contribution.

Common pharmaceutical excipients that are suitable for use as polymeric stabilizers include the cellulosics, such as hydroxypropylcellulose (HPC) and hydroxypropylmethylcellulose (HPMC), povidone (PVP K30), and pluronics (F68 and F127) [11,14–16]. The molecular weights of these polymers are usually between 50 k and 100 kDa. The chains should be long enough to provide a steric layer, but not too big to slow down dissolution. The surfactant stabilizers can be non-ionic, such as polysorbate (Tween 80), or anionic, such as sodium laurylsulfate (SLS) and docusate sodium (DOSS). Cationic surfactants are typically not used as stabilizers for oral formulation due to their antiseptic properties. Smaller surfactant molecules can also stabilize nanoparticles, but are usually more prone to Ostwald ripening and particle growth. Several groups have reported the use of the above stabilizers in their work [11]. Also, surfactants often help in the wetting and dispersion of the drug particles which are usually very hydrophobic. In marketed products based on Elan's NanoCrystal® technology, stabilizers such as HPMC E3, Povidone, HPC-SL, DOSS, and SLS have been used.

Nanosuspensions are typically converted to a solid dosage form for clinical formulations. Prior to drying, redispersants need to be added to the nanosuspension to ensure complete redispersion of nanoparticles into their primary, pre-drying state [17]. Sugars, such as sucrose, lactose, and mannitol, are commonly used as redispersants in oral formulations. The sugar molecules serve as "protectants" and prevent nanoparticles from aggregating as they are concentrated during drying [17].

3.2. Characterization of nanoformulations

A broad range of characterization tools and techniques exists for

coverage of these characterization tools. Techniques for characterizing nanoparticles can be generalized into two sub-groups. The first group deals with attributes and properties of single nanoparticles, such as their particle size and surface charge (zeta potential). Particle crystallinity, dissolution, and surface coverage also fall in this category. The second group measures bulk properties, such as the viscosity. Redispersibility testing is additionally used to evaluate the redispersion of solid nanoformulations in relevant media, such as water and gastric fluid.

The most basic property of a nanoparticle is its size. Various methods are available for particle size measurement [18]. A popular technique is laser light scattering, which allows quick determination of the particle size and distribution. Many models are available from Horiba [Irvine, CA, USA], Malvern [Worcestershire, UK], and Microtrac [Montgomeryville, PA, USA] *etc*. One model that has been frequently used is the Horiba LA-910, which can measure from 50 nm to 1000 μm. Typically, only a few drops of nanosuspensions are required for a measurement (note that dilution into an aqueous medium is necessary). Useful information includes the mean values, along with the D10, D50, and D90 (D90 means that 90% of the particles, by volume, are below the given size). The particle size distribution of milled nanosuspensions is typically narrow, with a coefficient of variation (CV) of about 25 to 40%. For comparison, the CV of latex standard particles, which are relatively monodisperse, is generally 5 to 10% whereas the CV of un-milled APIs, which are more polydisperse, is typically > 100%.

Another fundamental property of nanoparticles is their surface charge. Surface charges can arise from (i) ionization of the particle surface or (ii) adsorption of ions (such as surfactants) onto the surface. Typically, the surface charge is assessed through measurements of the zeta potential. Zeta potential is the potential at the hydrodynamic shear plane and can be determined from the particle mobility under an applied electric field [13]. The mobility will depend on the effective charge on the surface. Zeta potential is also a function of electrolyte concentration. Examples of dilution media are aqueous KCl solutions, *e.g.*, 10^{-4} M. Various models are available from Brookhaven [Worcestershire, UK], Horiba [Irvine, CA, USA], Malvern [Worcestershire, UK], Matec [Northborough, MA, USA] *etc*. The addition of anionic surfactants typically leads to a more negative zeta potential value. Zeta potential values in the −15 mV to −30 mV are common for well-stabilized nanoparticles.

Viscosity is one of the more prominent bulk properties. For a nanosuspension, whose viscosity can vary dramatically, depending on the extent of flocculation, it is helpful to determine the viscosity as a function of shear rate. Either a controlled-stress or a controlled-strain rheometer can be used (note that the yield stress can only be determined with the former design). Several models are available from TA Instruments [New Castle, DE, USA], Malvern [Worcestershire, UK], and Brookfield [Middleboro, MA < USA]. Typically, measurements can be made using the cone-and-plate geometry. The working shear rate range is from 0.01 to 1000 s^{-1}. The viscosity ranges from 1 cP for water or dilute nanosuspensions to 1000 cP or greater for concentrated nanosuspensions. Newtonian behavior (constant viscosity across the usual range of shear rates) is typical of well-stabilized nanosuspensions, while shear thinning (decreasing viscosity

3.3. Process development at lab and commercial scales

3.3.1. Feasibility of nanosuspension

At the early stage in development, the API is usually in tight supply, *e.g.*, even an amount of 100 mg may be hard to come by. Therefore, it is crucial that the feasibility of a nanomilled suspension can be assessed with as little API as possible. Typically, the feasibility work can be carried out at the small scale using 100 mg or less of API. The Nanomill® System [Elan Drug Discovery, King of Prussia, PA] can be employed. The working capacity of the smallest chamber is 10 mL, and a very small suspension volume can be evaluated. The nanomilling process involves the high shearing of drug suspensions in the presence of grinding media, as described by Merisko-Liversidge et al. [11]. The milling media is a highly cross-linked polystyrene resin (500-μm beads). Selected stabilizers can be screened, such as the cellulosics and pluronics. The batch milling time is normally within a few hours at a mill speed of 5000 rpm. The particle size of the milled suspension can be checked at the completion of milling. Drug suspensions with terminal mean particle size in the 100- to 250-nm range are generally deemed feasible and can be considered for preclinical pharmacokinetic evaluation (the 'success rate' in reaching the described mean particle size range, based on oral bioavailability enhancement is around 80% to 90% in our experience). The milled material can be recovered from the suspension + media mixture. At this juncture, only short-term physical stability (*e.g.* 24 to 48 h) needs to be demonstrated, mainly to cover the duration of the animal study. If oral bioavailability enhancement is achieved and further development is warranted, then additional formulation development and optimization work can be conducted.

While particle size and morphology of the starting API are of less concern if nanosizing is to be employed in formulation development, the chemical form of the API needs be considered prior to laboratory testing. Typically, the neutral form is the preferred starting form. While there has been an example of a salt form-containing nanoformulation, such as Par Pharmaceutical's MEGACE® ES with its acetate salt, pharmaceutical salts are generally not preferred. Possible liabilities of the salt form are (i) risk of disproportionation, *e.g.*, an HCl salt disproportionating to the free base form during nanomilling, (ii) risk of aggregation due to charge-based interactions in the small intestine, such as those with bile salts, and (iii) rapid solubilization and turnover of nanoparticles of a salt form into larger particles of the neutral form due to the pH changes in the gastrointestinal (GI) tract. Furthermore, the API's solubility should be low to minimize the potential for Ostwald ripening, and the most stable form in water should be used. APIs with ionizable groups and pKa between 2 and 7 (*e.g.* physiological pH range) run the risk of charge-based interactions even if not presented as a salt. The typical starting particle size of the API is between a few microns and a hundred microns. Larger starting materials are acceptable at the feasibility stage, but run the risk of clogging the nanomill at the larger processing scale (in which case, an API pre-milling step is usually employed).

Another consideration is the possibility of shear-induced API

often the cause of formation of amorphous form of the API, which can lead to enhanced solubility and Ostwald ripening. Oftentimes, poor physical stability can be attributed to amorphous form formation. One remedy would be to mill at a lower speed. Generation of heat by the milling process can also result in form conversion. The mill is typically jacketed to minimize the temperature rise. The crystallinity (and form) of the milled API can be checked by XRPD. This is accomplished by spinning down the nanoparticles *via* ultracentrifugation and performing a measurement on the moist sediment. Crystalline peaks of the API should be identifiable on top of the broad amorphous band of the polymeric stabilizer.

3.3.2. Nanosuspension for toxicology study

The next step in formulation development is geared toward supporting toxicology studies. An early-phase toxicology study typically spans 2 weeks to 3 months. The API requirement could range from a few hundred grams to a few kilograms. It is usually acceptable to formulate as a liquid nanosuspension at this stage [as opposed to a solid nanoformulation, which is preferred in a clinical setting (see Section 3.3.3)]. In the following subsections, various aspects in formulating and manufacturing nanosuspensions for toxicology studies are discussed: from composition and process to storage and manufacturing logistics.

3.3.2.1. Stabilizer selection. A nanosuspension formulation for toxicology studies needs to be stable as well as be processable at the target drug concentration. Hence, formulation screening work should continue until a sufficiently stable nanosuspension can be identified. The work can be conducted at the small scale, for example, with the Nanomill® system and at low drug concentrations. A reasonable stability protocol would be to mill various nanosuspensions and monitor the particle size of these suspensions at 5 °C and ambient, starting from a day to a few weeks. A two- to three-week time is adequate to identify a sufficiently stable nanosuspension. Typically, the search should produce a few feasible polymeric stabilizers, such as HPMC or HPC. A suitable working polymer:drug ratio is from 0.05:1 to 0.5:1. After a stable composition has been identified, the next step is to scale-up the drug loading to the target concentration. Normally, the target will be at least 100 mg/mL to meet the needs for toxicology studies. At these higher concentrations, the main issue is usually flocculation. Physical stability of more concentrated suspensions generally falls in line with those of dilute suspensions. The major consequences of flocculation are two-fold. The first is the larger effective particle size with reduced surface area for dissolution. The second is the viscosity increase (but this is generally minor and not problematic). In many cases, flocculation can be minimized by raising the level of anionic surfactant, such as SLS or DOSS, which helps improve wetting and electrostatic stabilization. Care should be taken not to add excessive surfactant as this can result in enhanced solubility and Ostwald ripening. To characterize a nanosuspension formulation, only a small quantity is needed, on the order of tens of grams of API. This low API requirement is advantageous, given the limited supply of API available at this

One concept that could prove useful in the selection of polymeric stabilizer is that of surface coverage. In principle, to fully provide steric stabilization, the polymeric stabilizers must fully adsorb onto the surfaces of the nanoparticles. While nanosuspensions can and have been formulated successfully, little attention is paid to the whereabouts of the stabilizer. Knowledge of the adsorption isotherm may help provide additional insights into the formulation efforts. Panmai and Deshpande [19] described a convenient method for determining the adsorption isotherm of a nanosuspension, which involves the determination of the fractions of the stabilizer that are bound to the drug surface and unbound in solution for a given polymer concentration. A drug example was given using HPC-SL and PVP K29/32 as stabilizers (mean particle size = 100 nm). First, a series of nanosuspensions were prepared for different stabilizers and at different amounts (ranging from 0.05:1 to 0.5:1 stabilizer: drug). Then, the nanosuspensions were ultracentrifuged to settle the nanoparticles, leaving a clear supernatant, which was assayed for the concentration of the unbound polymer. Through mass balance, the fractions of bound and unbound polymers were calculated. The resulting adsorption isotherm clearly showed a monotonic adsorption and surface saturation for HPC-SL. On the other hand, there was a virtual lack of surface adsorption for PVP K29/32. The greater affinity of HPC-SL is likely due to its greater hydrophobicity than that of PVP. Furthermore, the minimum ratio of HPC-SL to drug to ensure surface coverage is around 0.12 to 1. This result is very much in line with the common working ratios of 0.1:1 to 0.2:1 for stabilizer:drug. The value is expected to change with the stabilizer and the particle size. Hence, this approach may be used to select a polymeric stabilizer on a more rational basis.

3.3.2.2. Milling process. A conventional media mill would be needed to process the amount of API and suspension volume required for toxicology studies. Assuming a drug concentration of 100 to 200 mg/mL for a typical toxicology study (in dogs or rats) the required suspension volume is often greater than 5 L. One example of a suitable media mill is the Dynomill [Glen Mills, Inc., Clifton, NJ], with chamber sizes of 300 mL and 600 mL. Inside the milling chamber is a shaft with a series of impellers, which provide the high-shear agitation (up to 4000 rpm). Larger mills, such as the Netzsch mills [Netzsch Inc., Exton, PA], which come in 2-L, 10-L, and 60-L chamber sizes, also exist to handle even larger volume requirements. To supply a large volume, the mill is configured in the recirculation mode. For example, a 600-mL chamber, charged with milling media and suspension, can be connected to a vessel of 5 to 10 L. The suspension then flows into the milling chamber, undergoes intense media grinding, and exits the mill through a small gap. The milling media are strained by the gap and retained within the mill. The mill and the vessel are jacketed to control the temperature. The inlet suspension is around 5 to 10 °C while the outlet product can be anywhere from 15 to 30 °C, or even higher, depending on the mill speed and product viscosity. The overall milling time scales according to the residence time in the chamber. The required supply can typically be prepared within a

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